

A detailed microscopic image of plant cells, likely from a halophyte, showing thick cell walls and large central vacuoles. The cells are stained, with some showing prominent purple and yellow hues. The overall texture is granular and complex, typical of biological tissue at the cellular level.

MARIUS-NICUȘOR GRIGORE CONSTANTIN TOMA

STRUCTURI SECRETOARE DE SĂRURI LA HALOFITE

O ABORDARE INTEGRATIVĂ



EDITURA ACADEMIEI ROMÂNE

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SALT-SECRETING STRUCTURES OF HALOPHYTES.
AN INTEGRATIVE APPROACH

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(coperta I+IV: glande salifere, din epiderma inferioară a limbului foliar de la *Limonium gmelini*)

CUPRINS

Introducere.....	7
1. LOCUL ȘI ROLUL STRUCTURILOR SECRETOARE DE SĂRURI ÎN CADRUL STRATEGIILOR GENERALE ALE HALOFITELOR. PRECIZĂRI TERMINOLOGICE.....	11
2. DEFINIȚIA ȘI CLASIFICAREA STRUCTURILOR SECRETOARE DE SĂRURI.....	13
3. STRUCTURA ANATOMICĂ A FORMAȚIUNILOR SECRETOARE DE SĂRURI.....	21
3.1. Glande salifere la reprezentanți din familia <i>Plumbaginaceae</i>	21
3.2. Glande salifere la specii din familia <i>Tamaricaceae</i>	85
3.3. Glande salifere la specii ale genului <i>Frankenia</i>	101
3.4. Glande salifere la specii de mangrove.....	113
3.5. Glande salifere la specii din familia <i>Primulaceae</i>	135
3.6. Glande salifere la specii din familia <i>Poaceae</i>	143
3.7. Peri veziculari (salini) la specii din familia <i>Chenopodiaceae</i>	173
3.8. Celule epidermice veziculare (CEV) la specii din familia <i>Aizoaceae</i> (<i>Mesembryanthemaceae</i>).....	212
3.9. Structuri secretoare de săruri la specii din familia <i>Convolvulaceae</i> ...	231
3.10. Structuri secretoare de săruri la <i>Lavatera arborea</i> (familia <i>Malvaceae</i>)..	236
4. ORIGINEA ȘI EVOLUȚIA STRUCTURILOR SECRETOARE DE SĂRURI.....	243
Bibliografie.....	254
Index de familii, genuri și specii.....	277
Abstract.....	282

ABSTRACT

SALT-SECRETING STRUCTURES OF HALOPHYTES. AN INTEGRATIVE APPROACH

This book represents an anatomical monograph of one of the most important strategies of halophytes' life, namely, the secretion of salts in excess outside of body plant affected by high salinity in the soil. The work focuses primarily on reviewing the salt-secreting structures of almost all halophytes genera and species, where is well established that these adaptive mechanisms are present. First of all, it was necessary to discuss some very interesting and controversial theoretical issues regarding the terminology used for these secreting salts structures and their classification. Our intention at this level was to put order into the existing classifications by proposing a classification based on structural, anatomical criteria. This is because the language used abroad is not uniform, but heterogeneous and not based on anatomical criteria, but mainly on the functional one. There is no uniformity neither in salt-secreting structures definitions; we often found that there is confusion and even arbitrary in use of terms which are not synonymous, although the boundary between them is sometimes very fragile.

In the first part of the paper (**Place and role of salt-secreting structures in the context of general strategies of halophytes. Terminology background**) we have attempted to specify the place of salt-secreting structures between the major halophytes strategies. We have shown that they represent an important adaptation, which plays an active role in the adaptive strategies of halophytes. Moreover, the presence of these mechanisms defines a separate group within halophytes, the group of crynohalophytes. Salt-secreting structures are included, most often in the category of mechanisms limiting the content of salts (evasion mechanisms) in the plant body.

In the second part of the work (**Definition and classification of salt-secreting structures**) we have used the anatomical vision to put order into the existing definitions and classifications. The intention was not to far easier, because there is great structural and functional diversity among the salt secreting formations among the halophyte species. Sometimes, the foreign authors use the term "gland", sometimes the term "hydathode", and in some cases, the term "secreting trichome". Choosing the title of this book is based mainly to our intention to cover all the existing structural types.

We have adapted, modified and completed a previous definition of Fahn's (1988) regarding secretory tissues in vascular plants, using our anatomical vision in the phenomenon reviewed by us.

In this sense, the salt secreting structures comprise:

1. Structures eliminating salts into the vacuole, the situation of *Atriplex* and *Chenopodium* species. Salt is eliminated into a central vacuole of the bladder cell of the leaf trichomes. These cells are situated on top of narrow, 1- to 3-celled stalks. Growth of bladder cell is accompanied by the formation and expansion of a central vacuole.
2. Structures (glands, *stricto sensu*) eliminating salts outside of the cells. Here, may be included two types of glands:
 - a. bicellular and monocellular glands, mainly on *Poaceae* species;
 - b. multicellular glands, such as those of *Limonium*, *Tamarix*, *Avicennia*, *Frankenia*, *Cressa cretica*, *Lavatera arborea*.
3. Epidermal bladder cells of *Mesembryanthemum* species, which occupy an intermediary and special position between salt secretory structures. Despite the fact that some authors have included these bladder cells in the group of secretory trichomes, our opinion is that these cells must be regarded, histo-anatomically speaking, as special epidermal cells, which accumulate and eliminate salts outside of plant body.

The third chapter of the work (**Anatomical structure of salt-secreting devices**) represents indeed the proper and most consistent part of our work. It contains about 300 pages and 240 black and white and color figures. We are dealing these salt-secreting structures in an integrative manner and not in a purely descriptive one. It means that we tried to correlate these structures with their function, their adaptive value and ecological significance. In this regard, we have reviewed an impressive number of scientific papers (about 385), to which we added, naturally our results, related to *Atriplex tatarica*, *Halimione verrucifera* and *Limonium gmelini* taxa. These are, in fact, almost the only species of Moldova having salt secreting structures.

Thus it was discussed the structure of glands belonging to *Plumbaginaceae*, which secrete salts and, respectively, mucilages. The number of species possessing salt glands in this family is quite high; they vegetate in arid and salt habitats worldwide, and are relatively well studied from a structural and ultrastructural point of view. Glands may be composed of 8 or 16 cells, arranged in concentric layers (circles) on the surface of aerial organs. The best represented genera in this regard are *Limonium* (*Statice*), *Acantholimon*, *Plumbago*, *Armeria*, *Limoniastrum*.

The *Plumbaginaceae* salt glands are included in two categories:

1. Chalk glands (chalk secreting), well known as Mettenius or Licopoli organs, generally localized to or in some depressions of abaxial layer of leaves and stems. Sometimes, these glands are bounded by epidermal large cells groups or by trichomes. The proper

glands consist of 4 or 8 epidermal cells, arranged in prolonged layers, bounded by 1 or 2 layers, each one being formed by accessory cells. The walls between the secretory gland cells and surrounding cells are cutinized. The secreting “organs” of this type have been generally described as chalk glands because they exude deposits of calcium salts and water, calcium salts being sometimes scattered on the leaf or stem surface from raindrop. The amount of calcium salts secreted by these glands is in connection with soil nature, despite the fact that *Limonium* species investigated by de Fraine (1916) do not secrete chalky material.

2. Mucilage glands of some *Plumbaginaceae* species; those localized in the basal leaf axil and their upper surface of *Limonium bellidifolium* and *L. binervosum*, described by de Fraine (1916), consist in one cap cell, localized on a basis composed of cells with very thick walls, covered by a cuticle.

The results of our investigations on *Limonium gmelini* evidenced salt glands (“Licopoli organs”) in leaf structure, at the level of both epidermis, confirming the structure with 8 cells, described by other authors. In addition, we also observed these glands in the leaf petiole, but at this level, they are more rarely.

The salt glands of *Tamaricaceae* occur especially on *Tamarix* species. These comprise 6 secreting cells, with dense cytoplasm and large nucleus and 2 extraglandular, collecting cells, intensely vacuolized. Well studied glands were those of *T. aphylla*, *T. pentandra*, *T. ramosissima* and *T. mannifera*. Ultrastructural investigations showed that the gland consists in three pairs of secretory cells separated by thin cell walls; the secretory cells have many mitochondria, microvacuoles and cell wall protuberances. The gland is enclosed by cuticle, except for the regions termed “transfusion zones”. The cuticle extends inward along the lateral sides of the gland and it ends at the transfusion zone. The cell walls of the transfusion zone are confluent with the walls of the underlying cells. We commented extensively on this level, the implications of these glands of *Tamarix* species in some ecological imbalances that occur due to soil salinization following secretory activity of these taxa. On this occasion, we defined a new type of salinization, along with traditional types; it’s about phytosalinization as a direct result of salt secretion by *Tamarix* species. Other *Tamaricaceae* species having salt secreting glands are: *Myricaria germanica*, *Reaumuria oxiana*, *R. fruticosa*, *R. hirtella*.

Frankeniaceae is another family having many species which possess salt glands, especially belonging to *Frankenia*. These structures have been intensely studied, both structural and ultrastructural. They are relatively homogenous, structurally speaking. The gland consists of 6 secretory cells, which secrete a saline solution, accumulated on leaf surfaces and leached by rain. Species having salt glands are *F. floribunda*, *F. pulverulenta*, *F. laevis*, *F. intermedia*, *F. reuteri* and *F.*

patagonica. Ultrastructural studies show that *Frankenia* salt glands consist in a bilateral complex of six secretory cells. This cells aggregate is almost completely enclosed by cuticle. The only part of the gland boundary which is cuticle-free represents a portion of the wall between each inner secretory cell and the neighboring mesophyll cell; this region has been termed the transfusion zone. On this species, this zone has many plasmodesmata. The most peculiar aspect of the transfusion zone of *Frankenia* is the occurrence of bulbous enlargements in the wall.

Mangroves are coastal ecosystems comprising halophyte species which have salt glands, especially occurring as glandular trichomes. These glands are common on *Avicennia*, *Aegialitis* and *Acanthus* species. The most intensely studied glands are those of *Avicennia* species. These glands are localized on both surfaces of the leaf. On the upper surface, they are more numerous, occurring isolated on the basis of some epidermis invaginations. The cap of gland consists of 8 columnar short cells and the stalk is formed by a single discoidal cell; this cell is localized on the top of 8 small, modified epidermal cells. On the lower surface, the glands are relatively numerous; they differ from those of the upper surface by the fact that they are not sunken, consisting of a tetracellular head and having a single large epidermal cell, at the base. The mature gland is covered by cuticle, which does not allow the transport of salt by apoplastic pathway. Salt flows through the basal cell (often called collecting cell) then through stalk and secretory cell and finally is excreted in a subcuticular space in the terminal part of gland. During the warming and dry days, on the leaf surface salt crystals are formed due to the drying of secreted solution. In the early morning when humidity is high, salt excreted from the leaf surface absorb hygroscopic water, which dissolves the salt, which can drop the leaves in the form of salted droplets.

Primulaceae is represented by two genera having salt glands: *Glaux* and *Samolus*; these have a relatively simple structure, in regard with other salt glands. Ordinarily, the glands are located at the level of some depressions in the structure of foliar limb.

Poaceae, a great and heterogeneous family, also includes many salt-secreting halophytes. They mainly have a bicellular structure, but some species may have even a single-cell gland structure, thus being the simplest configuration of salt glands, recorded on *Porteresia coarctata*. The glands of a large number of species have a two-celled hairlike structure, with a basal cell and a cap, secreting cell. These glands have been intensely studied, as many species of *Poaceae* have an increased economic value and high phytoremediation capacity; in these circumstances a good knowledge of the biology of these species it is an obligatory issue in this regard. The simplest type of these glands is the two-celled hairlike found in *Aeluropus littoralis*, formed by a large basal cell and a small cap cell with cutinized walls. As in the case of *Spartina*, the gland is not sunken in the

epidermis, but relatively located above it. Its structure is rather similar to that of a hair than that of a gland.

A three-celled gland is found in *Chloris gayana*, and is comprised of a large collecting cell, a stalk one and an upper one, which is assumed to be the secreting one.

The ultrastructural investigations showed that *Spartina* salt gland consists in two cells, a large basal cell and a smaller one, a dome-shaped cap cell which is located on a neck-like protrusion of the basal cell. The basal cell is sunk in the leaf tissue. The top of the cap cell is usually positioned with or below the level of the epidermis outer surface, and a cylindrical depression occurs between the cap cell and other four adjacent epidermal cells. The basal cell, cap cell, and the four adjacent epidermal cells form the salt-gland complex. A cuticular layer occurs on the walls of all the cells of the salt-gland complex which are exposed to the atmosphere. The cuticle covering the cap cell is continuous with the cuticle covering the neck-like protrusion of the basal cell and the lateral walls of the epidermal cells. Toward the top of the cap cell the cuticle forms a distended, dome-like covering. The cells of the salt-gland complex which are in contact with mesophyll cells are not covered with cuticular layer.

Chenopodiaceae includes *Atriplex* and *Chenopodium* species having vezicular hairs (salt hairs); these have a structure somewhat different from that of the proper gland, because the excess of salt is accumulated in the large central vacuole of vezicular cell of the hair. Salts are consequently released to the outside by collapsing of cell walls, thus being deposited on the surface of aerial vegetative organs and then removing by rain or dew water drops.

As a general configuration, an *Atriplex* salt hair is comprised of two cells: a small stalk cell and a large bladder cell. The stalk cell structure shows great similarities to various cells of salt glands. It is composed of thick cytoplasm rich in mitochondria, dense endoplasmic reticulum, and numerous small vesicles; the salt hairs also contain chloroplasts. Stalk cells are connected to adjacent bladder or mesophyll cells by numerous plasmodesmata.

It has been shown that salt content and concentration of sodium and chloride in bladders are higher than that of mesophyll cells, and certainly of the external solution. Sodium content usually exceeded that of the chloride. The bladders also exhibit a higher electronegativity with respect to the rest of the mesophyll cells and to the external solution. Hence the direction of salt movement is from the medium through the mesophyll into the bladders. Since this process is against a concentration gradient, it is probably required a metabolic energy.

The ultrastructural studies evidenced that the salt hair is comprised of many cells consisting the stalk of the hair and a bladder cells, which is proeminent to the leaf epidermis. It is assumed that when the vacuole of bladder cell is filled with saline solution, the cells are collapsing thus eliminating the ions in excess. It seems that the hair is covered by a cuticular layer. The secretory hairs of *Atriplex* are

included in a special category, covered by a cuticle and the lateral walls of the stalk cells being entirely cuticularized and/or suberified.

Mesembryanthemum crystallinum (Aizoaceae) presents large epidermal bladder cells on the shoot surface. These serve as an external water reservoir and contribute therefore to salt accumulation and its subsequent removal. They have been rather studied ecophysiologically and in a less extent, anatomically.

It was suggested that epidermal bladder cells of *M. crystallinum* are a xerophytic adaptations, because they represent single water storage cells, being a more rarely features than proper water storage tissues.

These living aqueous cells always remain filled with protoplasm and cell-sap; they never contain air. The volume of water that they contain, however, varies between wide limits. When transpiration is slack, they may be gorged with water, for instance at night or in dull weather, but during strong transpiration they supply the assimilating cells with water, and then collapse strongly.

Thus, bladder cells of *M. crystallinum* have a clear function in salt adaptation; they became bigger, covering the shoot surface, as the plants are subjected to salt treatments.

Convolvulaceae has two species with salt glands, less studied than those of other halophyte species: *Cressa cretica* and *Ipomoea pes-caprae*. Their structure reminds those of glands belonging to *Plumbaginaceae*, *Tamaricaceae*, *Frankeniaceae* and mangroves.

Salt glands of *Lavatera arborea* (Malvaceae) seems to be the last discovered salt secreting structures, being therefore, less studied. The gland of *Lavatera* consists of a large basal cell, two middle cells and five top cells. The top cells are capped by a dome-shaped cuticle which encloses a subcuticular space. The basal cell is characterized by a large central vacuole which is surrounded by a dense cytoplasm, with a smooth endoplasmic reticulum and some vesicles. Its cytoplasm is linked with the adjacent lower middle cell via plasmodesmata.

A final part of the paper (**The origin and evolution of salt-secreting structures**) constitutes a completely new and original chapter regarding the approach of these structures. Our interest was mainly related to the evolution of secretory mechanisms and not that of species, so we made some assumptions and evolutionary scenarios, with reference to possible pathways of appearance and evolution of glands.

From an evolutionary point of view, it seems that here were selected multiple a different histo-anatomical lines (profiles), if we refer to the general configuration of the glands:

1. *Multicellular glandular structures* line, seen in species belonging to the *Plumbaginaceae*, *Tamaricaceae*, *Frankeniaceae*, *Avicenniaceae*, *Primulaceae*, *Convolvulaceae* (*Cressa cretica* and *Ipomoea pes-caprae*) and *Malvaceae* (*Lavatera arborea*);

2. *Mono-cellular structures line* (*Porteresia coarctata*) and *bicellular line*, represented in other *Poaceae* species;
3. *Vezicular (accumulating, secreting) hairs line* from *Chenopodiaceae* taxa;
4. *Epidermal bladder cells line* of *Aizoaceae* (*Mesembryanthemum* sp.).

It is possible that histo-anatomical adaptations of halophytes to salinity took place over time, in an *integrated, successive, hierarchical and topographic* way, at the level of axial and lateral vegetative organs. This means that these plants have possessed and still possess in the same time several mechanisms regulating the content of salts, such as metabolic, physiological, genetic and molecular, in addition to histo-anatomical mechanisms. We cannot certainly state when and how the first mentioned mechanisms have occurred. It is more likely that some of them have appeared on the bases of some existing anatomical structures. Regarding the ways in which the secretory structures had appeared and evolved in any point of their evolution, we can only give some hypothesis. It is therefore very likely that salt glands (in the wide sense), to be derived from:

1. **Normal epidermal cells**, as a result of *signal*, probably mediated by hormonal way, as a response to a stressful factor: salinity and/or excessive aridity. Studies regarding the ontogenesis of these secretory structures generally support this hypothesis. Epidermal cells could then undergo successive divisions that led to the building of salt-secreting structures in the configuration that we know today. Secretory function could be achieved by increasing metabolic activity through development of vacuole, mitochondria and nucleus activity. The secretory function being gained, it would have been maintained, as a general law of evolution states that an acquisition made by a living organism can not be lost. Evolution is a complex irreversible phenomenon. It is likely that coastal plants subjected by aerial parts to salt spray to be offered such a response more quickly than inland halophytes, because the action of stressful agent would have been quicker and more direct;

2. **Guard cells**, which represented *already* epidermal cells (topographically speaking) more evolved and specialized than the remaining cells from the epidermis level. At least, they would have supplied or accompanied salt secretion, due to the characteristic configuration. Firstly, the pore could be involved in this mechanism. This is the stomatal opening assuring and regulating the rate of transpiration and gaseous exchange between the atmosphere and the internal air spaces. Secondly, there were substomatal and suprastomatal cavities, which would have managed to accumulate, even temporarily, the salts. This is an interesting hypothesis, and the possibility of salt excretion *via* stomata it is not entirely excluded.

3. **Trichomes (hairs)**, arising at epidermal surface, which could initially have a protection function, especially against sunlight. This

supposition would be related therefore to the case of *Poaceae* secretory structures. At first glimpse it seems a plausible assumption if we are thinking on the *Poaceae* salt glands, viewed sometimes as secreting *microhairs*, having monocellular and often, bicellular structure. We must say, as an argument that here is sometimes a difficulty in nowadays, to distinguish between these types of secretory structures and other types of trichomes. This might be because salt secretion becomes prominent and salt whiskers occur abundantly only when plants are exposed to saline treatments. Such observations are very exciting because we believe that this scenario would have happened in the course of halophytes evolution: as the environmental salinity increased, trichomes have acquired a secretory function, ensuring the removal of salt excess outside of plant body. It seems to be rather a *diffuse* secretory function in this heterogeneous group, because the *Gramineae* salt glands are not structurally very complicated. In addition this may be correlated with the fact that the *Poaceae* have also other mechanisms regulating the salt content in the tissues. In this point of discussion, we suggest a new idea. It seems that at any group of plants, having many *compensatory* mechanisms (physiological and metabolic, especially) for salt regulation, the anatomical adaptations are less defined. Moreover, it was shown that, generally, the secretion is more intensely as the concentration of salts in the environment is higher. However, some salt-secreting *Poaceae* which would have migrated into non-saline environments, have been ceased to remove the salt *via* salt glands, losing therefore this mechanism, ineffectively in the new glycophytic conditions.

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